

FSUM 10302
PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of Robert A. Holton et al.
Serial No. 09/063,477
Filed April 20, 1998
For PROCESS FOR THE SELECTIVE DERIVATIZATION OF TAXANES
Examiner Ba K. Trinh

TO THE COMMISSIONER OF PATENTS AND TRADEMARKS

SIR:

DECLARATION OF ROBERT A. HOLTON UNDER 37 C.F.R. 1.608(b)

I, Robert A. Holton, declare and state as follows:

1. I am a Professor of Synthetic Organic, Biorganic, and Organometallic Chemistry at Florida State University in Tallahassee, Florida and am an inventor of "Process For The Selective Derivatization Of Taxanes" which represents the subject matter of the present application.
2. As disclosed in the present application, Zhuming Zhang, Paul A. Clarke and I invented a process for the selective derivatization of taxanes. The process for selectively derivatizing the C10 position of the taxane described in claims 1-14 and on page 4, line 19 to page 9 of the specification was invented under my direction.
3. The invention was conceived and reduced to practice before May 21, 1997, the filing date of U.S. Patent No. 5,874,595. The reduction to practice was accomplished by Zhuming Zhang conducting the "Attempt to protect C(7)OH by cbz" experiment as documented on laboratory notebook page 45 (Exhibit A). 53 mg of dibenzyl dicarbonate was added to 3 mg of 10-deacetyl baccatin III in tetrahydrofuran solvent at room temperature and the mixture reacted overnight. A proton NMR of the final reaction mixture revealed the unexpected product 10-benzoyloxy baccatin III in greater than 90% yield.
4. An aspect of the invention wherein baccatin III is produced, described in claim 3 of the present application, was also conceived and reduced to practice before May 21, 1997. The reduction to practice was accomplished by Zhuming Zhang when conducting the "Attempt to

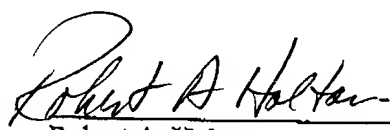
protect C(10) H by (CH₃CO)-O" experiment as documented on laboratory notebook page 49 (Exhibit B). Ten microliters of acetic anhydride was added to 3 mg of 10-deacetyl baccatin III in tetrahydrofuran solvent at room temperature. An additional 100 microliters of acetic anhydride was added and the mixture reacted overnight. A proton NMR of the final reaction mixture revealed the baccatin III product in greater than 80% yield.

5. A further aspect of the invention as described in claim 6 of the present application was also conceived and reduced to practice before May 21, 1997. The reduction to practice was accomplished by Zhuming Zhang when conducting the "Generation of baccatin III from 10DAB" experiment as documented on laboratory notebook page 67 (Exhibit C). One ml of acetic anhydride was added to 14 mg of 10-deacetyl baccatin III and 3.5 mg ZnCl₂ in tetrahydrofuran solvent at room temperature. A proton NMR of the final reaction mixture revealed baccatin III as the major product.

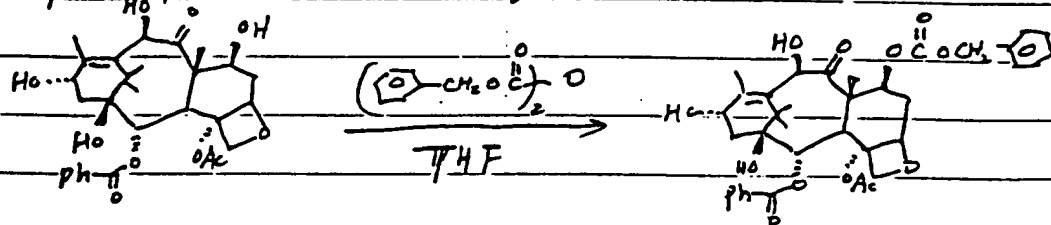
6. Therefore, on the basis of prior invention I believe that we are *prima facie* entitled to judgment relative to U.S. Patent No. 5,874,595 within the context of 37 C.F.R. §1.608(b).

7. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

8/22/00
Date


Robert A. Holton

Attempt to protect C(7) OH by Cbz



Materials used

(1) 10 DAB

(2) dibenzyl dicarbonate
(97%)

(3) THF

FW

544

286.29

AMT

3 mg (0.00551 mmol)

53 mg (38%, 0.984 mmol)

0.5 mL

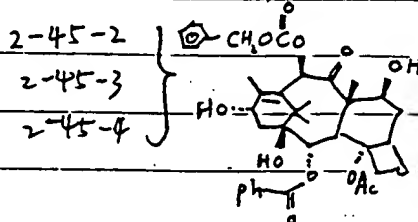
Procedure: same as Page 44, except THF was used in stead of CH_2Cl_2

The rxn proceeded smoothly, TLC indicated the formation of product. The rxn was left for overnight.

Medium 1:9	Medium 1:9
0.5 h	overnight

This rxn almost go to completion,
very, very promising !!

$^1\text{H NMR}$ @ 2-45-1 mixture of crude

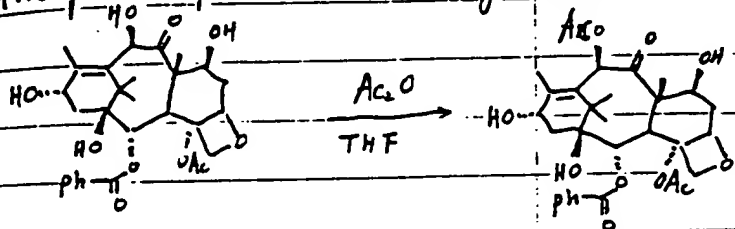


so Cbz attached to C(10) OH

What a surprise ???!

high yield is obtained ($\geq 90\%$)

Attempt to protect C(10)H by $(\text{CH}_3\text{C}(=\text{O}))_2\text{O}$



Materials used

FW

AMT

(1) 10 DAB

544

3 mg (0.00551 mmol)

(2) Ac_2O

102.09

10 μL (20 μg , 0.11 mmol)

$d = 1.082$

(3) THF

0.5 mL

Procedure: the rxn of 10DAB with $(\text{PhCH}_2\text{OC}(=\text{O}))_2\text{O}$ suggested that similar reaction could take place between 10DAB + Ac_2O

To a solution of 10DAB in THF was added Ac_2O under Nitrogen.

The reaction mixture was stirred at room temperature and monitored by TLC. TLC indicated the slow process of the rxn and a faint amount of product formed. At this stage it is not clear

whether the rxn is proceeding or not. So 100 μL more Ac_2O was added. After 2h,

TLC

visible rxn was observed, a single spot show up in high R_f value. After overnight, $\geq 80\%$ conversion was

observed. At this stage, this rxn was stopped by evaporating the solvent away and taken by ^1H NMR

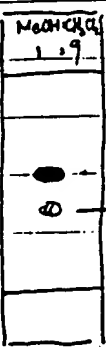


0.5h



2h

1.5h after more Ac_2O was added



20h

crude ^1H -NMR 2-49-1 indicated $\geq 80\%$ Baccatin III + 10DAB + small amount of (7-Ac²10DAB) (by evaporating the solvent?)

2-49-2 pure Baccatin III.

Procedure: To a THF solution of 10DAB + $ZnCl_2$ was added Al_2O_3 under N_2 . The solution was stirred at room temperature and monitored by TLC.

actually 18 mg ZnCl_2 (may have a little bit of H_2O)

Diagram illustrating the execution timeline of a program with three instructions, each having an effective address (EA) of Hex 8:1 and a start time (SM).

- Instruction 1:** EA: Hex 8:1, SM (Start Time).
- Instruction 2:** EA: Hex 8:1, SM (Start Time).
- Instruction 3:** EA: Hex 8:1, SM (Start Time).

The timeline shows the progression of time from 0.5h to 1.5h, with each instruction starting at its respective time point.

¹H NMR 2-67-1 crude mixture
(too dilute)

$$\text{H}_2\text{O} \cdot \text{CH}_2\text{O}$$

2-67-2 - crude mixture

Major baccatin III + small
amount 7thD-diacetyl
+ oxetanering opened
product.

Low temperature experiment is recommended.